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Small Dense LDL Cholesterol is a Promising Biomarker for Secondary Prevention in Elder Men with Stable Coronary Artery Disease

A short running title **Small Dense LDL for Secondary Prevention in Men (**40 characters**)**

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STRUCTURED ABSTRACT (237 words)

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Abstract 237 words

Aim: The study objective was to investigate whether small dense LDL cholesterol (sdLDL-C) was superior to LDL-C and other biomarkers to predict future cardiovascular events (CEs) in secondary prevention.

Methods: sdLDL-C measured by a homogeneous assay, remnant lipoprotein cholesterol (RLP-C), LDL particle diameter (LDL-PD), and other biomarkers were compared in 345 men ≥65 years of age with stable coronary artery disease (CAD). Baseline LDL-C was 100.5 ± 30.1 mg/dL. CEs were CEs, including cardiovascular death, onset of acute coronary syndrome, need for arterial revascularization, hospitalization for heart failure, surgery procedure for cardiovascular disease, and hospitalization for stroke were monitored for 5 years.

Results: CEs occurred in 96 patients during the study period. LDL-C, sdLDL-C, non-HDL-C, apolipoprotein B, RLP-C, glucose, HbA1c, and BNP were significantly higher; LDL-PD and apolipoprotein A-1 were significantly lower in patients with than in those without CEs. Age-adjusted Cox regression analysis showed that sdLDL-C per 10 mg/dL but not LDL-C was significantly associated with CEs (HR 1.206, 95% CI 1.006–1.446). A significant association of sdLDL-C and incident CEs was observed in statin users (HR 1.252, 95% CI 1.017–1.540), diabetes patients (HR 1.219, 95% CI 1.018–1.460), patients without diabetes (HR 1.257, 95% CI 1.019–1.551), and hypertriglyceridemia (HR 1. 376, 95% CI 1.070–1.770).

Conclusions: sdLDL-C was the most effective predictor of residual risk of future CEs in stable CAD patients using statins and in high-risk CAD patients with diabetes or hypertriglyceridemia.

Key words Coronary artery disease, Diabetes mellitus, Secondary prevention
Small dense LDL, Statins

INTRODUCTION

Low-density lipoprotein cholesterol (LDL-C) is currently considered as the most important target for reducing cardiovascular risk. LDL includes fractions of large buoyant and small dense particles[1)]. Small dense LDL (sdLDL) particles are thought to be more atherogenic than large buoyant LDL (lbLDL) because of higher penetration of the arterial wall, lower binding affinity for the LDL receptor, prolonged plasma half-life, and lower resistance to oxidative stress [1-3)]. Increased sdLDL cholesterol (sdLDL-C) represents an increase in the number of atherogenic LDL particles because sdLDL particles are smaller and contain less cholesterol.

The HDL Atherosclerosis Treatment Study reported a positive association of the plasma concentration of sdLDL particles and progression of coronary artery stenosis that was confirmed by gradient gel electrophoresis (GGE), vertical auto profile ultracentrifugation, nuclear magnetic resonance spectroscopy, and ion mobility[4)]. In our cross sectional studies, heparin magnesium precipitation was used to demonstrate that high sdLDL-C, not high lbLDL-C, concentration was closely associated with the angiographic and/or clinical severity of coronary artery disease (CAD) independent of classical coronary risk factors [5,6)]. Our previous cohort study found that increased sdLDL-C and the sdLDL-C/LDL-C ratio were significantly associated with an elevated risk of cardiovascular events (CEs) in patients with stable CAD[7)], and prospective population-based cohort studies in Japan and the USA reported that increased sdLDL-C was significantly associated with the incidence of CAD independent of LDL-C [8-10)].

A meta-analysis of 61 prospective cohort studies showed that age substantially

attenuated the positive relationship between total cholesterol and CAD mortality[11]. The INTERHEART study, a worldwide case-control study including more than 25,000 participants, found a significant decline in the odds ratio of myocardial infarction (MI) for each change of one standard deviation (SD) in total cholesterol, LDL-C, non-high-density lipoprotein cholesterol (non-HDL-C), and apolipoprotein B (apoB) with increase in age[12]. The association of sdLDL-C with the risk of CE recurrence in elderly patients with CAD has not been compared with that of LDL-C. The study aim was to determine whether sdLDL-C is a better predictor than LDL-C and/or other lipid biomarkers of future CEs in elderly patients with stable CAD.

METHODS

Study Patients

This observational cohort study included of 345 consecutive male patients ≥65 years of age with angiographically confirmed CAD at Showa University Hospital between September 2003 and December 2011. At the time the study began enrolling patients, aggressive lipid-lowering treatment was not common in patients with CAD. Significant CAD was defined as a 50% or greater narrowing of the diameter of one or more coronary artery branch on an arteriogram, and/or a prior history of percutaneous coronary intervention (PCI), and/or coronary artery bypass surgery (CABG). The peripheral artery disease (PAD) was defined as the Ankle Brachial Index (ABI) of less than 0.90 using an oscillometric device (Form/ABI, Colin Company, Ltd., Komaki,

Japan) and/or history of endovascular treatment. Patients with acute coronary syndrome (ACS), nephrotic syndrome, renal dysfunction (serum creatinine >1.5 mg/dL), severe hepatic disease, infectious disease, currently treated for malignancy, on hemodialysis, taking drugs for thyroid dysfunction, or with any other serious condition were excluded. Patients who could not be followed for 3 months after coronary angiography were also excluded. The institutional review board of Showa University approved the study protocol, which was registered at UMIN-CTR (UMIN000027504). The investigation conformed to the ethical principles of the Declaration of Helsinki, and informed consent was obtained from all subjects.

Baseline Evaluation

Fasting blood samples were obtained by venipuncture immediate before cardiac catheterization. The LDL particle diameter (LDL-PD) and serum lipid biomarkers, except sdLDL-C, were assayed within 3 days of sampling; unused samples were stored −80ºC. Of the 345 participants, 245 men had a past history of MI, 272 had previously undergone PCI and/or, CABG, 234 were taking lipid-lowering drugs, and 102 men experienced coronary revascularization because of coronary angiography findings. Hypertension was determined by the medical history or by a pressure above 140 mmHg systolic or 90 mmHg diastolic [13]. Diabetes mellitus was defined as a fasting serum glucose value >126 mg/dL, glycated hemoglobin (Hb) A1c values estimated by (=1.019×HbA1c [Japan Diabetes Society] + 0.3) greater than 6.5%, and/ or the current use of medication for diabetes[13]. Dyslipidemia was defined as the current use of

lipid-lowering medications and/or meeting the criteria of the Japan Atherosclerosis Society for fasting serum lipid levels, i.e., LDL-C ≥140 mg/dL, HDL-C <40 mg/dL, or TG ≥ 150 mg/dL [13]. A serum creatinine–based estimate of glomerular filtration rate (eGFR) was calculated as follows: eGFR = 194 x $Cr^{-1.094}$ x $Age^{-0.287}$ (x0.739 for women)[14]. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Patients with a reported smoking habit of at least one cigarette per day on admission were classified as current smokers.

Definition of CEs

In-hospital medical records were evaluated 60 months after the baseline blood screening. The endpoints were: (1) the onset date of the first CE; (2) the date of death; and (3) the date of the patient's last visit to Showa University Hospital. CEs were defined as death caused by CVD, onset of ACS, need for coronary or other arterial revascularization including restenosis after PCI; hospitalization for heart failure; surgery for any CVD; and hospitalization for ischemic or hemorrhagic stroke. Coronary revascularization within 3 months was not considered a CE because at that time, unexpected and scheduled coronary revascularization could not be distinguished.

Lipoprotein and Brain Natriuretic Peptide Assays and Inflammatory Markers

Total cholesterol, triglycerides, HDL-C, HbA1c, apolipoproteins, and lipoprotein(a) were assayed by standard laboratory procedures. RLPs were isolated from serum by immunoaffinity mixed gels containing anti-apolipoprotein A1 (apoA1) and

anti-apoB100 monoclonal antibodies (Japan Immunoresearch Laboratories, Takasaki, Japan). The cholesterol concentrations of the unbound fraction were measured as RLP-C[15]. Serum LDL-C was determined by a direct homogenous assay using detergents (LDL-EX, Denka Seiken, Tokyo, Japan). Serum samples were kept frozen at −80ºC until used for a direct homogenous assay for sdLDL-C as previously described [16]. The LDL-C and sdLDL-C assay kits were provided by Denka Seiken. LbLDL-C was estimated by subtracting the sdLDL-C concentration from the LDL-C concentration. The sdLDL-C/LDL-C ratio was also calculated from direct measurements. lbLDL-C values estimated by this method have previously been correlated with values determined by ultracentrifugation[16]. Non-HDL-C was estimated by subtracting the HDL-C concentration from the total cholesterol concentration, and peak LDL-PD was determined by 2%–16% nondenatured polyacrylamide gel electrophoresis as described by Nichols, et al.[17]. High sensitivity C-reactive protein (hsCRP) was assayed by the Dade Behring BN method[18]. Plasma brain natriuretic peptide (BNP) was measured by radioimmunoassay.

Statistical Analysis

Statistical analysis was performed using the SPSS 23.0 software package (SAS Institute, Cary, NC, USA). The baseline characteristics of patients with or without CEs during follow-up were compared using Wilcoxon tests, because most of the variables did not have a Gaussian distribution. Categorical variables were compared by chi-square tests. Although this was a case-control study, cumulative incidence was estimated by the

Kaplan–Meier method in patients stratified by the median sdLDL-C level. The date of the baseline lipid measurements was set as the landmark point from which cardiovascular outcomes were evaluated. Clinical data obtained from patients who were lost to follow-up were censored, and were used for the period for which follow-up was available. Age-adjusted Cox regression analysis of the patient variables was used to calculate hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) of the risk of future CEs. Variables that differed significantly in patients with and without CEs were included in a multivariate analysis. All statistical analyses were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Baseline Characteristics and CEs

The 5-, 3-, and 1-year follow-up rates were 75.5%, 84.8%, and 94.6%, respectively, and first-time CEs were observed in 96 (27.8%) of the 345 patients (**Supplementary Table 1**). Eleven patients died of CVD, (one of an acute MI, six of heart failure, and four of unknown cause). Eighty-five (24.6%) were hospitalized with nonfatal CVDs, 11 (3.2%) with ACS, 11 (3.2%) with congestive heart failure, and 14 (4.05%) with ischemic stroke. Forty-six (13.3%) underwent revascularization and three (0.9%) received endovascular treatment. No patients were hospitalized with hemorrhagic stroke. Comparison of the baseline characteristics of patients with and without CEs (**Table 1**) revealed a significantly higher prevalence of diabetes and PAD, greater need for coronary

revascularization at baseline, and fewer statin uses among those who experienced a CE. A comparison of laboratory findings (**Table 2)** found that the mean LDL-C was about 100 mg/dL that was lower than that in a previously studied cohort[7]. The sdLDL-C in patients treated with statins were significantly lower than those untreated with statins (26.1 ± 11.5 vs 31.5 ± 14.2, p=0.003). LDL-C, sdLDL-C, non-HDL-C, LDL/HDL-C ratio, apoB, RLP-C, glucose, HbA1c, and BNP were significantly higher, and LDL-PD and apoA-1 were significantly lower in patients with CEs. The differences in lbLDL-C, HDL-C, eGFR, and hsCRP in the two groups were not significant.

Correlation of LDL-C and SdLDL-C and Patient Characteristics

The Spearman rank-order correlation of LDL-C and sdLDL-C and patient characteristics are shown in the **Supplementary Table 2**. Although sdLDL-C is a part of LDL-C, sdLDL-C had stronger correlations with biomarkers of atherogenic dyslipidemia such as triglycerides and RLP-C than LDL-C.

Kaplan–Meier Event-Free Survival Analysis and Cox Regression Analysis

The Kaplan-Meier event-free survival curves among patients above or below the median levels for sdLDL-C in patients, diabetic patients and patients treated with statins are shown in **Figure 1**. Sixteen patients who died of causes other than CVD and without experiencing any nonfatal CEs, were treated as censored cases. Patients with sdLDL-C ≥ 25mg/dL were significantly increased risk for CEs (log-rank 6.155, p=0.003). Similar

results were observed in diabetic patients and patients treated with statins at baseline (log-rank 6.492, p=0.011 and log-rank 4.193, p=0.041, respectively). Age-adjusted Cox regression analysis (**Table 3**) revealed that increases in LDL-C, sdLDL-C, non-HDL-C, LDL-C/HDL-C, apoB, glucose, HbA1c, and BNP, and decreases in LDL-PD and apoA1 were significantly associated with an increased risk of CEs; RLP-C was not. Multivariate analysis showed that decreased LDL-PD and increased HbA1c were significantly associated with CEs (**Table 3, Model 1**). Both LDL-C and sdLDL-C had significant positive correlations with non-HDL-C and apoB, and we found a negative correlation between sdLDL-C and LDL-PD. When LDL-C, sdLDL-C, and apoA1 among lipid markers were included in the multivariate model, sdLDL-C per 10 mg/dL was independently associated with the risk of CEs (HR 1.210, 95% CI 1.003–1.459) (**Table 3, Model 2**). The age-adjusted Cox regression analysis conducted in the study subpopulations is summarized in **Table 4**. Significant associations of sdLDL-C and occurrence of CEs were observed in various patient populations including patients treated with statins, those with or without diabetes, or those with hypertriglyceridemia. Both LDL-C and sdLDL-C were significantly associated with CEs in diabetes patients, but the significance was lost following multivariate analysis. The HRs were 1.063 (95% CI 0.945–1.196) for LDL-C and 1.129 (95% CI 0.889–1.433) for sdLDL-C.

Discussion

To the best of our knowledge, this is the first study to report that elevated sdLDL-C (determined by an automated homogenous assay) was independently associated with

CE recurrence in elderly patients with stable CAD. These results are consistent with our previous obtained in 190 patients with stable CAD using precipitation assay of sdLDL-C concentration[7]. In the previous study, Cox's proportional hazard analysis failed to find an association of increased sdLDL-C with the risk of CEs [7]. In this study, multivariate Cox regression analysis showed that decreased LDL-PD was significantly associated with the occurrence of CEs independent of LDL-C and sdLDL-C, which is evidence in support of sdLDL as atherogenic LDL particles. The measurement of LDL-PD by GGE requires a separation time of >24 hours, which makes it unsuitable for use in clinical practice. The direct homogenous assay for sdLDL-C does not require any pretreatment and can be performed with the chemistry auto analyzers routinely used in clinical laboratories[19]. The study found a significant association between sdLDL-C and the occurrence of CEs in the entire study population and in high-risk subpopulations including diabetes patients and those with hypertriglyceridemia. The results confirm that sdLDL-C, compared with LDL-C, was a more important biomarker for secondary prevention.

A meta-analysis of eight randomized controlled statin trials, found that on-treatment levels of non-HDL-C were more strongly associated with the risk of major CEs than the levels of LDL-C and apoB among statin-treated patients [20]. A study of recent ACS patients treated with statins reported that fasting triglycerides at the initial randomization predicted short- and long-term risk of major CEs[21]. Both publications suggest that the cholesterol content of triglyceride-rich lipoproteins poses a residual risk in patients receiving effective statin therapy. A correlation of RLP-C and sdLDL has

also been shown[22], and Kugiyama *et al.* demonstrated that patients in the highest RLP-C tertile (> 5.1 mg/dL) in a group of 135 CAD patients had a higher incidence of CAD events than those in the lowest tertile (≤ 3.3 mg/dL), even though their LDL-C levels were less than 100 mg/dL[23]. In a later study of 190 patients treated with statins after ACS, they found that a high RLP-C (≥ 5.4 mg/dl) was a significant risk of secondary events independent of conventional risk factors (HR 2.94; 95% CI: 1.40–6.18; $p < 0.01$)[24]. This study, which compared LDL-C, sdLDL-C, RLP-C, non-HDL-C, and apoB in patients with stable CAD, found that only sdLDL-C was independently associated with CEs in patients treated with statins. A recent study of the LDL-C reduction achieved by statins with or without ezetimibe in patients with ACS found a greater reduction of sdLDL-C in patients with coronary plaque regression (measured by ultrasound) than in those with plaque progression[25]. The evidence from a variety of sources thus supports sdLDL-C as a promising biomarker of residual risk in patients currently treated with statins.

The Japanese Elderly Diabetes Intervention Trial found a positive association of LDL-C with incident coronary events. Both HbA1c and non-HDL-C were positively associated with stroke[26]. Cox regression analysis found that in this study, LDL-C, sdLDL-C, apolipoprotein B, and HbA1c were significantly associated with CEs in diabetes patients, and the HR for sdLDL-C was slightly higher than those for LDL-C and apoB. However, the association between these lipid biomarkers and CEs was no longer significant in the multivariate analysis. Future studies in large populations are required to investigate lipid biomarkers in diabetes patients with CAD. On the other

hand, the association of increased sdLDL-C and non-HDL-C, and decreased in LDL-PD with CEs in patients without diabetes are in agreement with results of the Multi-Ethnic Study of Atherosclerosis. That study reported significant associations of sdlDL-C and incident CAD in patients with normal fasting glucose, not in those with impaired fasting glucose or diabetes mellitus[9].

This study has several limitations including a small sample size and low follow-up rate. Secondly, the CEs included those related to unstable plaque as well as revascularization for restenosis after PCI, atherogenic cardiovascular diseases, and ischemic heart failure requiring hospitalization. It remains unclear whether elevated sdLDL-C are related to ischemic heart failure. In addition, hospitalization of heart failure might be due to non-atherosclerotic disease, although all patients in the present study had coronary atherosclerosis. Thirdly, the effects of lipid-lowering therapy and target levels of sdLDL-C could not be investigated. Fourthly, lipid-lowering treatment was not evaluated during follow-up. Fifthly, left ventricular ejection fraction, frailty, malnutrition, and anemia were not captured in the analysis. Future prospective studies should thus be conducted to evaluate these issues in larger samples.

In conclusion, in elderly patients with stable CAD, elevated sdLDL-C levels measured by an automated homogenous assay were strongly associated with CE recurrence independent of LDL-C. Secondly, decreased LDL-PD was significantly associated with incident CEs independent of LDL-C and sdLDL-C. Thirdly, of all the lipid biomarkers, only sdLDL-C was independently associated with CEs among patients treated with statins. Fourthly, a significant association of sdLDL-C and incident CEs

was consistently observed all patient subpopulations including those with diabetes and hypertriglyceridemia, which are well-known high-risk populations. The results showed sdLDL-C to be a more effective secondary prevention biomarker than LDL-C to predict future CEs. A large cohort study is required to determine the appropriate target level of sdLDL-C.

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Disclosure statement

Yasuki Ito is an employee of Denka Seiken. The other authors have no conflicts of declare.

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Figure Legend

Figure 1. The Kaplan–Meier event-free survival of patients stratified by the median sdLDL-C concentration (25 mg/dl): (A) All patients; (B) Patients treated with statins; (C) Diabetes patients.

Supporting Information Legend

Supplementary Table 1. Cardiovascular events

Supplementary Table 2. Spearman's correlation of LDL-C or sdLDL-C and patient characteristics

Table 1. Baseline clinical characteristics

	Whole (n=345)	CE (n=96)	non-CE (n=249)	*p*
Age, years	72.8 ± 5.4	72.9 ± 5.2	72.7 ± 5.5	0.597
Body mass index, kg/m^2	23.8 ± 3.1	24.5 ± 3.3	23.5 ± 3.0	0.018
Prior MI, n (%)	205 (59.4)	56 (58.3)	149 (59.8)	0.446
Prior PCI/CABG, n (%)	272 (78.8)	72 (75)	200 (80.3)	0.174
Need for coronary revascularization at baseline	102 (29.6)	43 (44.8)	59 (23.7)	<0.001
Peripheral Artery Disease	31 (9.0%)	15 (15.6%)	16 (6.4%)	0.011
Risk factors				
Hypertension	262 (75.9)	72 (75)	190 (76.3)	0.451
Diabetes mellitus	115 (33.3)	48 (50)	67 (13.5)	<0.001
Dyslipidemia	301 (87.2)	86 (89.6)	215 (86.4)	0.269
Smoking (Current and Former)	267 (77.4)	78 (81.3)	189 (75.9)	0.179
Cardiovascular medication				
Calcium Channel Blocker	149 (43.2)	50 (52.1)	99 (39.8)	0.026
ACE inhibitors	191 (55.4)	59 (61.5)	132 (53.0)	0.098
ARB	50 (14.5)	8 (8.3)	42 (16.9)	0.028
Beta-blocker	102 (29.6)	28 (29.2)	74 (29.7)	0.516
Antiplatelet	311 (90.4)	89 (92.7)	222 (89.5)	0.283
Oral anti-diabetic drugs	94 (27.2)	38 (39.6)	56 (22.5)	<0.001
Insulin	26 (7.5)	13 (13.5)	13 (5.2)	0.009
Statin	208 (60.3)	49 (51.0)	159 (63.9)	0.02
Lipid-lowering except statin	26 (7.5)	7 (7.3)	19 (7.6)	0.559

Data are means ± SD or number (%). Risk factors and medications, including prior lipid-lowering drugs at baseline, were evaluated at the time of blood sampling. N/A = not available; ACE-I = angiotensin-converting enzyme inhibitor; ACS = acute coronary syndrome;

ARB = angiotensin II type 1 receptor blocker; CABG = coronary artery bypass graft surgery;

MI = myocardial infarction; PCI = percutaneous coronary intervention.

Table 2. Laboratory findings in patients with and without CEs.

	Whole (n=345)	CE (n=96)	non-CE (n=249)	*p*
LDL-C, mg/dL	100.5 ± 30.1	107.3 ± 30.0	97.9 ± 29.7	0.008
sdLDL-C, mg/dL	28.2 ± 12.9	31.8 ± 15.1	26.9 ± 11.7	0.008
lbLDL-C, mg/dL	72.2 ± 24.2	75.5 ± 25.3	71.0 ± 23.7	0.171
LDL-PD, Å	256.2 ± 4.5	255.3 ± 4.9	256.5 ± 4.2	0.032
Non-HDL-C, mg/dL	128.7 ± 34.0	137.7 ± 35.3	125.3 ± 32.9	0.003
Triglycerides, mg/dL	123.1 ± 70.5	133.7 ± 87.6	119.0 ± 62.5	0.195
HDL-C, mg/dL	46.6 ± 14.4	44.1 ± 12.7	47.5 ± 15.0	0.060
LDL-C/HDL-C	2.4 ± 1.0	2.6 ± 1.0	2.3 ± 1.0	0.001
sdLDL-C/LDL-C	0.28 ± 0.10	0.30 ± 0.12	0.28 ± 0.96	0.15
ApoA1, mg/dL	124.9 ± 25.8	119.2 ± 23.1	127.0 ± 26.5	0.013
ApoB, mg/dL	85.8 ± 21.0	91.0 ± 20.3	83.9 ± 21.1	0.004
Lipoprotein(a), mg/dL	22.8 ± 24.7	25.0 ± 25.2	22.0 ± 24.5	0.296
RLP-C, mg/dL	4.4 ± 3.0	4.8 ± 3.3	4.3 ± 2.9	0.04
Glucose, mg/dL	114.1 ± 35.5	121.3 ± 45.6	111.3 ± 30.5	0.038
HbA1c, %	6.3 ± 1.1	6.7 ± 1.5	6.1 ± 0.88	<0.001
eGFR, mL/min/1.73 m^2	62.4 ± 18.1	62.6 ± 20.4	62.3 ± 17.2	0.479
BNP, pg/mL	105.1 ± 170.7	149.0 ± 254.0	87.7 ± 119.5	0.021
hsCRP, mg/dL	0.40 ± 1.0	0.52 ± 1.4	0.35 ± 0.81	0.347

Table 3. Age-adjusted hazard ratios (95% CI) for occurrence of major CEs

variables	Univariate model	Multivariate model	
		Model 1	Model2
LDL-C /10mg/dl	1.085 (1.017-1.157)*	1.174 (0.977-1.410)	1.022 (0.937-1.114)
sdLDL-C /10mg/dl	1.256 (1.091-1.446)**	1.158 (0.903-1.486)	1.210 (1.003-1.459)*
LDL-PD	0.945 (0.905–0.987)**	0.933 (0.882–0.986)*	—
T-C /10mg/dl	1.054 (0.997-1.115)	—	—
Non HDL-C /10mg/dl	1.085 (1.026-1.148)**	1.129 (0.910-1.401)	—
LDL-C/HDL-C	1.340 (1.115–1.611)**	0.646 (0.313–1.355)	—
Apolipoprotein A1 /10mg/dl	0.892 (0.820-0.971)**	0.839 (0.694-1.016)	0.897 (0.822-0.980)*
Apolipoprotein B /10mg/dl	1.134 (1.034-1.243)**	0.782 (0.531-1.150)	—
RLP-C	1.035 (0.980–1.093)	—	—
Glucose	1.006 (1.001–1.011)*	0.996 (0.989–1.003)	—
HbA1c	1.407 (1.237–1.600)***	1.420 (1.143–1.765)**	—
BNP	1.001 (1.000–1.002)**	1.001 (1.000–1.002)	—

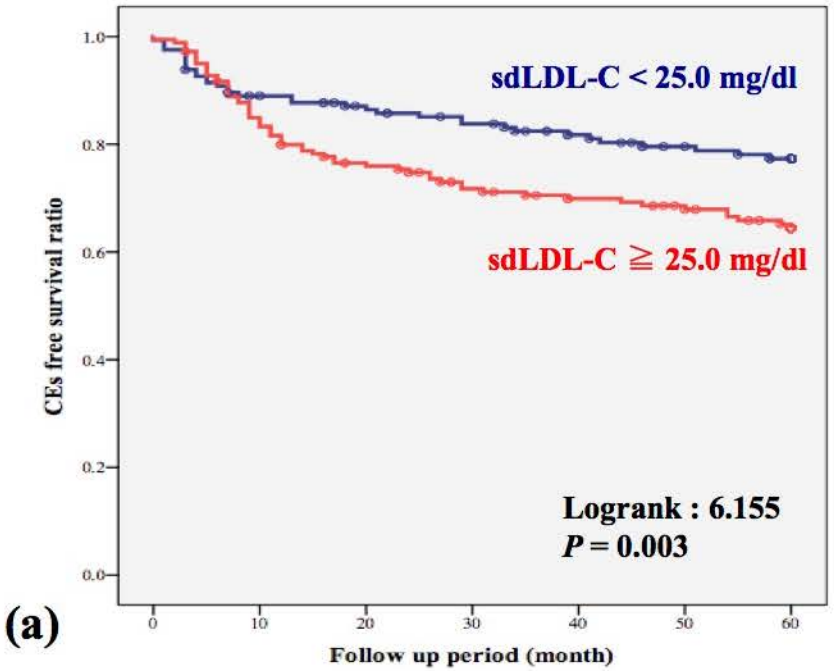
T-C = total cholesterol

*p<0.05, **p<0.01, ***p<0.001

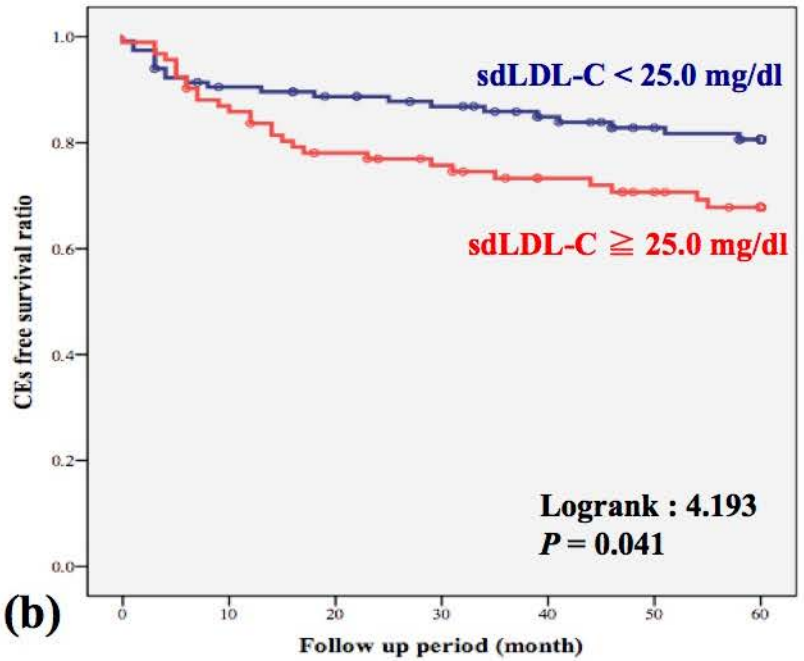
Table 4. Univariate age-adjusted hazard ratios (95% CI) for occurrence of major CEs in patient subpopulations

	Statin users (n=208)	Hypertriglyceridemia (n=80)	Diabetes (n=115)	Non-diabetes (N=230)
LDL-C /10mg/dL	1.062 (0.957-1.177)	1.089 (0.977-1.214)	1.103 (1.006-1.208)*	1.078 (0.985-1.180)
sdLDL-C /10mg/dL	1.252 (1.017-1.540)*	1.376 (1.070-1.770)*	1.219 (1.018-1.460)*	1.257 (1.019-1.551)*
sdLDL-C/LDL-C	6.111 (0.531–70.294)	5.453 (0.294–101.292)	3.455 (0.299–39.923)	5.888 (0.394-88.098)
LDL-PD,	0.956 (0.901–1.015)	0.966 (0.884–1.056)	0.960 (0.897–1.028)	0.934 (0.883–0.989)*
lbLDL-C /10mg/dL	1.016 (0.893-1.156)	1.037 (0.913-1.176)	1.088 (0.968-1.223)	1.058 (0.943-1.186)
Triglycerides /10mg/dL	1.005 (0.965-1.048)	1.029 (0.991-1.068)	1.003 (0.974-1.034)	1.044 (0.998-1.092)
T-C/10mg/dL	1.043 (0.958-1.136)	1.060 (0.968-1.162)	1.047 (0.972-1.128)	1.058 (0.977-1.146)
HDL-C /10mg/dL	0.932 (0.745-1.167)	0.877 (0.635-1.211)	0.895 (0.733-1.093)	0.842 (0.667-1.063)
Non-HDL-C /10mg/dL	1.060 (0.971-1.0157)	1.088 (0.987-1.200)	1.079 (0.996-1.169)	1.082 (1.001-1.170)*
LDL-C/HDL-C	1.281 (0.909–1.805)	1.222 (0.860–1.737)	1.394 (1.018–1.910)*	1.325 (1.037–1.692)*
Apo A1 /10mg/dL	0.919 (0.813-1.039)	0.886 (0.763-1.028)	0.894 (0.788-1.015)	0.907 (0.807-1.018)
Apo B /10mg/dL	1.079 (0.933-1.247)	1.104 (0.935-1.305)	1.157 (1.011-1.324)*	1.126 (0.993-1.277)
Lipoprotein(a)	1.004 (0.995–1.013)	1.023 (1.008–1.039)**	1.012 (1.000–1.025)	1.004 (0.994–1.015)
RLP-C	0.999 (0.899–1.111)	1.005 (0.918–1.100)	1.000 (0.936–1.068)	1.089 (0.940–1.261)
Glucose	1.005 (0.997–1.013)	1.000 (0.991–1.009)	1.002 (0.996–1.008)	0.995 (0.975–1.015)
HbA1c	1.574 (1.264–1.960)***	1.186 (0.916–1.534)	1.304 (1.088–1.563)**	1.157 (0.654–2.046)
eGFR	0.999 (0.981–1.018)	0.984 (0.962–1.007)	1.003 (0.988–1.018)	1.007 (0.989–1.025)
hs-CRP	0.424 (0.113–1.588)	0.892 (0.418–1.904)	0.949 (0.635–1.408)	1.202 (1.016–1.422)*
BNP	1.001 (0.999–1.003)	1.001 (0.999–1.003)	1.001 (1.000–1.002)**	1.001 (0.999–1.003)

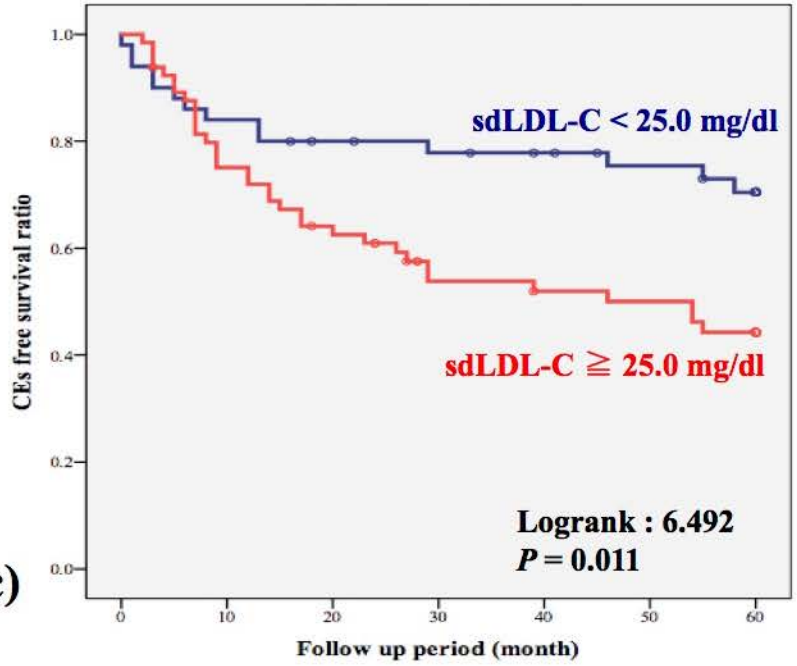
*p<0.05, **p<0.01, ***p<0.001


sdLDL-C < 25.0 mg/dl
sdLDL-C ≧ 25.0 mg/dl
Logrank : 6.155
P = 0.003
CEs free survival ratio
Follow up period (month)

(a)


sdLDL-C < 25.0 mg/dl
sdLDL-C ≧ 25.0 mg/dl
Logrank : 4.193
P = 0.041
CEs free survival ratio
Follow up period (month)

(b)


sdLDL-C < 25.0 mg/dl
sdLDL-C ≧ 25.0 mg/dl
Logrank : 6.492
P = 0.011
CEs free survival ratio
Follow up period (month)

(c)

Supplementary Table 1. Cardiovascular events

Cardiovascular events	Number
Fatal events	11
Acute coronary syndrome	1
Congestive heart failure	6
Other cardiovascular death	4
Nonfatal events	85
Acute coronary syndrome	11
Any revascularization (endovascular treatment)	46 (3)
Congestive heart failure	11
Stroke	14

Supplementary Table 2. Spearman's correlation of LDL-C or sdLDL-C and patient characterisrics

	LDL-C		sdLDL-C	
	Rho	*p*	Rho	*p*
age	−0.003	0.956	−0.091	0.092
BMI	0.092	0.092	0.155	0.004
LDL-PD	−0.119	0.029	−0.376	<0.001
Large LDL-C	0.902	<0.001	0.314	<0.001
Non-HDL-C	0.905	<0.001	0.758	<0.001
HDL-C	−0.086	0.11	−0.16	0.003
Triglycerides	0.165	0.002	0.452	<0.001
Apolipoprotein A1	−0.175	0.001	−0.078	0.158
Apolipoprotein B	0.886	<0.001	0.796	<0.001
Lipoprotein(a)	0.134	0.018	−0.025	0.661
RLP-C	0.335	<0.001	0.516	<0.001
Glucose	−0.117	0.03	0.024	0.66
HbA1c	−0.051	0.341	0.036	0.507
eGFR	0.036	0.499	0.098	0.069
hsCRP	0.156	0.004	0.124	0.022
BNP	0.011	0.85	−0.072	0.196